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Effect of CO_2 enrichment and elevated temperature on methane emissions from rice, *Oryza sativa*

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Abstract

Methane emissions from rice grown within Temperature Gradient Greenhouse Tunnels under doubled CO_2 concentrations were 10–45 times less than emissions from control plants grown under ambient CO_2. For two cultivars of rice (cvs. Lemont and IR-72), methane emissions increased with a temperature increase of 2°, from outdoor ambient temperatures to the first cell of the ambient CO_2 tunnel (ambient temperature + 2 °C). Within both tunnels and for both cultivars methane emissions decreased with further temperature increases (from 2° to 5 °C above ambient). Carbon dioxide enrichment stimulated both above- and below-ground production. Our original hypothesis was that increased CO_2 would stimulate plant productivity and therefore stimulate methane emission, since direct linkages between these parameters have been observed. We hypothesize that CO_2 enrichment led to the attenuation of methane production due to increased delivery of oxygen to the rhizosphere because of increased root biomass and porosity. The increased root biomass due to elevated CO_2 may have more effectively aerated the soil, suppressing methane production. However, this study may be unique because the low organic content (< 1%) of the sandy soils in which the rice was grown created very little oxygen demand.

Keywords: carbon dioxide, methane, porosity, rice, temperature, wetlands

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Introduction

The accumulation of methane in the present-day atmosphere is a well-documented component in the overall topic of global change and atmospheric chemistry (Chapellaz *et al.* 1990; Rasmussen & Khalil 1986; Steele *et al.* 1992; Dlugokencky *et al.* 1994, 1998). However, knowledge regarding the factors which control this phenomena, and how these factors will respond to changing global conditions, remains insufficient.

Of the major sources of methane, natural wetlands and rice paddies are the largest. Together they constitute 40–50%of the total flux of methane to the atmosphere (Cicerone & Oremland 1988). Projected increases in atmospheric levels of carbon dioxide (Rotty & Marland 1986; Trabalka *et al.* 1986; Kohlmaier *et al.* 1987) and mean global temperature (Moore & Bolin 1987; Donner & Ramanathan 1980) have the potential to affect wetland ecosystems and, consequently, their rates of methane emission.

Studies of natural and artificial wetlands have reported positive correlations between methane emission rate and plant above-ground biomass (Whiting & Chanton 1992; Whiting *et al.* 1991; Sass *et al.* 1990), CO_2 exchange (Whiting & Chanton 1993; Chanton *et al.* 1997), and root biomass (Sass *et al.* 1990). It is well documented that CO_2 fixation rates and phytomass accumulation will be enhanced by elevated CO_2 (Luxmore 1981; Baker *et al.* 1992; Kimball *et al.* 1983, 1986; Rogers *et al.* 1992, 1994; Curtis *et al.* 1990). The CO_2 effect may be particularly important for the enhancement of below-ground plant biomass. Furthermore CO_2 effects may be compounded by increased temperature as Idso *et al.* (1987) determined that a 3 °C temperature increase could significantly amplify the growth enhancement effect of carbon dioxide enrichment.

On the microbial level, methanogens generally respond to increased temperature with higher rates of

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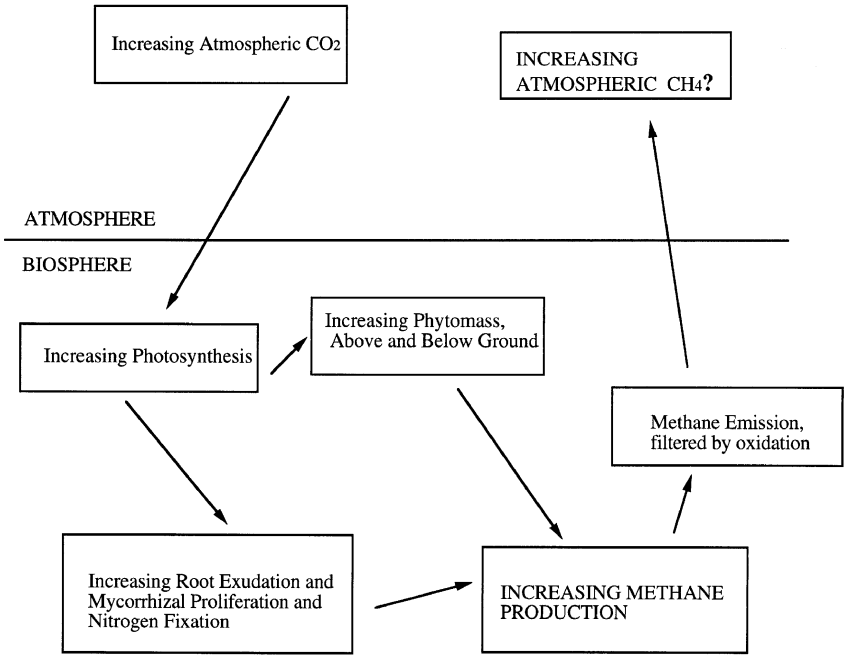

Fig. 1 Simple diagram showing the hypothesized linkage between increasing atmospheric CO_2 concentrations and CO_2 fixation rates, phytomass accumulation (Luxmoore 1981) and methane emission. Our work (Whiting *et al.* 1991; Chanton *et al.* 1993; Happell *et al.* 1993; Whiting & Chanton 1993, 1992) has demonstrated a direct linkage between net ecosystem exchange of CO_2, phytomass and CH_4 emission rates, so a feedback may exist whereby increasing concentrations of atmospheric CO_2 lead to increasing CH_4 emissions from natural and agricultural wetlands. These emissions may be attenuated by methane oxidation.

methane production (Kelly & Chynoweth 1981; Crill *et al.* 1988; Wilson *et al.* 1989; Zeikus & Winfrey 1976). There is evidence, therefore, to suggest that a positive feedback may exist whereby increasing concentrations of atmospheric CO_2 coupled with increased temperatures lead to greater rates of methane emissions from wetlands, thus exacerbating global warming. This hypothesized relationship is illustrated in Fig. 1.

The objective of this study was to assess the combined effects of increased temperature and double-ambient CO_2 levels on methane emissions from an agricultural wetland, specifically, two cultivars (Lemont and IR-72) of rice, *Oryza sativa L.* We hypothesized that increased CO_2 and temperature effects on plant and microbial communities would be increased methane emission rates. Greater CO_2 uptake rates and plant biomass were expected in response to the elevated CO_2 as well. We tested our hypotheses by making regular measurements of methane emission and CO_2 exchange as described in Chanton & Whiting (1995). Economic constraints and facility designs have limited the number of plants for a single experiment used in studies of plant response to coincident increases in temperature and carbon dioxide. This study was conducted using two Temperature Gradient Chambers (TGC) (Sinclair *et al.* 1995) at the United States Department of Agriculture research facility in Gainesville, Florida. The tunnel like TGCs represent a marked improvement over previously used facilities because they allow the manipulation of a large number of plants under a temperature gradient divided into discrete cells as temperature increases down the light transparent tunnel. The temperature increase is driven by solar heating of air pulled down the tunnel by a fan at the bottom end. At night or on cloudy days the temperature gradient is maintained by electric heaters.

Previous studies examining the effects of increased CO_2 on plant methane emissions are sparse. Dacey *et al.* (1994) and Drake (1992) reported CO_2 enrichment to increase methane emissions in a *Scirpus olneyi* marsh. They judged this increase to be sufficient to influence atmospheric chemistry. Hutchin *et al.* (1995) found a similar effect for cores of mire peat and vegetation under conditions of increased carbon dioxide concentration. Allen *et al.* (1994) found the combined effect of increased CO_2 and temperature for rice grown in outdoor, controlled environment plant growth chambers to be an increase in methane emissions. Preliminary results generated in a greenhouse by G. Whiting (pers. comm. 1996) show similar enhancement of CH_4 emission under elevated CO_2 in *Sagittaria*, a freshwater wetland macrophyte.

Materials and methods

Site description

All field work was conducted at the United States Department of Agriculture research facility on the University of Florida campus. Rice, *Oryza sativa* (cvs. Lemont and IR-72) was planted in two TGC and one

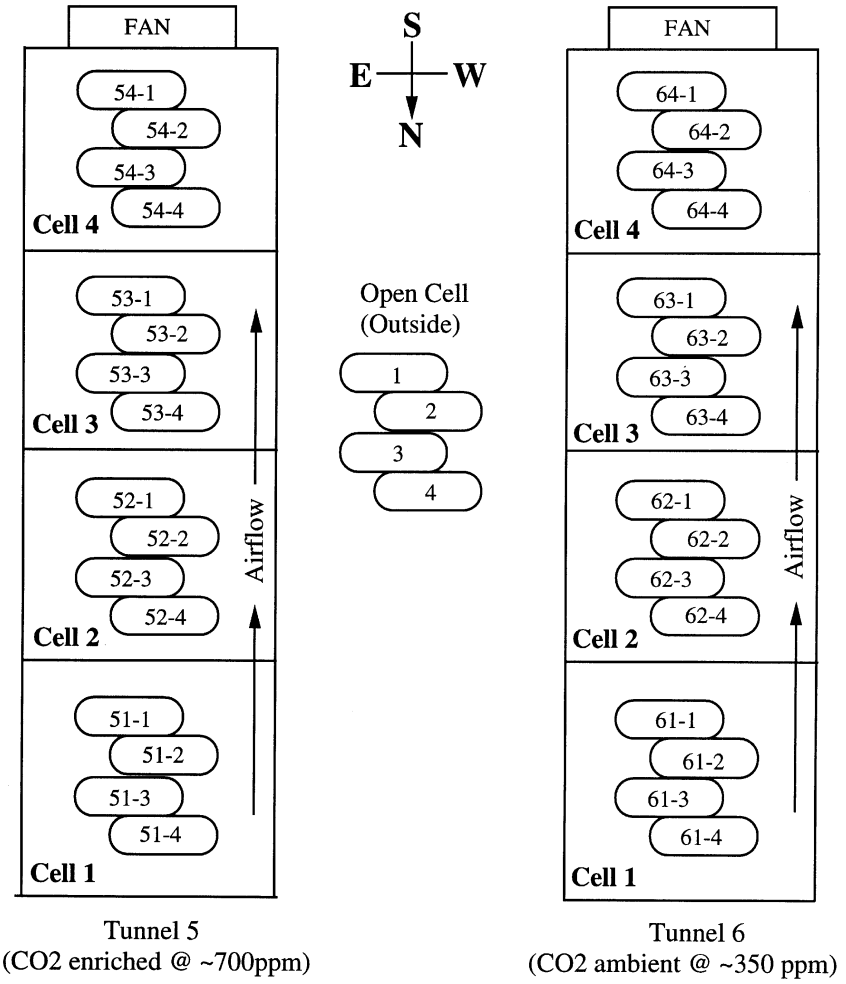

Fig. 2 Diagram of tunnels, open cell, and vat arrangement. An example of the tunnel vat numbering scheme is 51–1 which indicates Tunnel 5, Cell 1, Vat 1. The Open Cell was located outside, between the two tunnels. These four vats are referred to as Open 1 through Open 4. Vats 1 and 2 in each cell are cv. Lemont, 3 and 4 are cv. IR-72.

outside plot (Open Cell) on 15 June 1994. These TGC are 27.4 m long free-standing greenhouse tunnels composed of a polyethylene film supported by semicircular aluminium rods (Sinclair *et al.* 1995). A temperature gradient divided into four discrete cells was maintained within the tunnels by solar heating and electric heaters. The gradient varied from 2° to 5° C above ambient temperature down the tunnel from Cell 1 to Cell 4 (Fig. 2). Ambient temperatures typically reached the upper 30 s in the afternoon and dropped into the 20 s at night. A large variable speed fan at the end of each tunnel and small heaters down the sides were adjusted by computer according to conditions at any given time to maintain the gradient. Homogeneous temperature conditions were maintained within each cell by means of an overhead paddle fan which mixed the air in a given cell.

The carbon dioxide level in one tunnel was maintained at 350 ppm above ambient, or ≈700 ppm during the daytime throughout the growing period of the rice. This was accomplished by injection of CO_2 at the tunnel entrance as air was pulled in from outside. Up to 25 July (40 DAP, Days after Planting) CO_2 injection was controlled by adjusting the injection valve manually. During this period CO_2 was elevated and typically ranged from 550 ppm to 1000 ppm. Once computer control was established the CO_2 concentration was maintained within ≈50 parts of 700 ppm. In a second tunnel carbon dioxide was maintained at ambient levels by simply pulling outside air through the tunnel. As a control group 4 vats of rice organized like a given tunnel cell were maintained between the two greenhouses. This control group is referred to as the Open Cell.

Each TGC cell, including the Open Cell, contained 4 separate galvanized steel stock-watering troughs, or vats, which were 150 cm long, 60 cm wide, and 60 cm deep. The vats were filled to within 15 cm of the top with Arredondo fine sand (loamy siliceous hyperthermic Grossarenic Paleudult) taken from the top layer (topsoil) of the surrounding field. Vats in the ambient CO_2 tunnel were filled just prior to planting for these experiments,

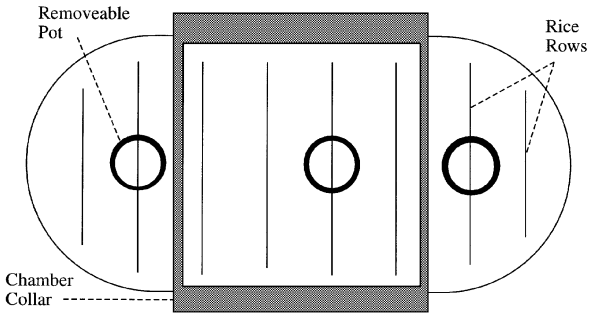

Fig. 3 Diagram of individual vat.

while the vats in the CO_2 enriched tunnel had been filled one year earlier, with a crop of rice grown during that time. This crop was harvested near ground level, then crowns were uprooted leaving some root material. One might have suspected that this difference in treatment would result in higher concentrations of organic matter in the soil of the vats within the CO_2 enriched tunnel and therefore greater rates of methane emission from this tunnel, all other things being equal. As will be shown below, although the level of organic matter for vats in the CO_2 enriched tunnel was higher ($0.70\% \pm 0.14$, $N = 14$ vs. $0.50\% \pm 0.16$, $N = 15$) than in the ambient tunnel (Schrope 1995), this was not the case. Analysis of soil pH and zinc levels (Schrope 1995) did not reveal any important differences between the soil in the two tunnels.

Vats 1 and 2 of each cell were planted with cv. Lemont, and vats 3 and 4 with cv. IR-72. Rice was planted at 262 plants per m^2 on 15 June and flooded on 24 June. Before planting, 6.78 g per m^2 of P and K were added. Nitrogen (14.9 g $N\,m^{-2}$) was applied as urea (45% N) at 8, 19, 44, 62, and 123 DAP. Each vat contained 3 removable pots (Fig. 3) which were the same height as the soil level with diameters of 15.24 cm. These were removed sequentially and sampled destructively to quantify above and below-ground biomass in terms of dry weight. An aluminium chamber base was in place at the centre of each vat for the duration of the study for mounting of the sampling chambers. The pots within these collar areas were not removed until after methane sampling had been completed for cv. IR-72. The middle pots in the cv. Lemont vats were not removed. The plants within the collar area of Lemont vats were instead maintained in order to allow study of a ratooned crop. A ratoon crop is a second-generation yield obtained from regrowth of plants following their clipping. Ratooned plants were clipped at 5 cm above the soil surface on 1 October 1994 (107 DAP) to allow the resulting regrowth crop from new tillers to be studied. Vats were partially drained at this point and reflooded on 17 October (123 DAP). The growing season for a ratooned crop is typically shorter than a normal season.

Gas exchange

Methane and carbon dioxide exchange were measured using closed phytochambers (Whiting *et al.* 1992; Chanton & Whiting 1995) which were attached to the collars in each vat. The chambers were rectangular and ranged in height from 50 to 140 cm. The open bottom had a 3-cm lip with a neoprene gasket to allow attachment to the collars. The soil area sampled was 56 cm by 66 cm. During sampling the chamber gasket was sealed to the collar using clamps. All sides of the chamber were clear, with three sides composed of transparent Teflon film. The front side and top were made of rigid polycarbonate, allowing transmittance of $\approx 90\%$ of incident PAR.

Temperature within the chamber was monitored by means of a mounted thermometer, and regulated to within 1 °C of the cell being sampled by controlling the rate of flow of cold water through a heat exchanger. Once sealed, six air samples were taken at four minute intervals using 60 mL syringes to pull air from the chamber head space through a sampling tube attached to the front of the chamber. Between replicate measurements chambers were vented. Samples were analysed for methane within a few hours of sampling using a gas chromatograph equipped with a flame ionization detector. Methane emission was determined 7–12 times for each plot during the regular and ratooned (second crop) growing seasons.

In order to allow comparison between seasonal methane emissions in each cell, an estimate was derived from individual vats. Daily values for the first 89 DAP (including an assumed value of 0 on the day of flooding, 9 DAP) in each vat were plotted on line graphs of uniform axes, and three copies were made. The area under each line was cut and weighed, and the three replicates averaged. This value was compared against the weight of a template to yield a seasonal rate of emission for each vat. Values for both vats of like cultivar in each cell were then averaged to allow comparisons between given treatment conditions.

At four points during the regular growing season measurements were made of CO_2 exchange (Whiting *et al.* 1992). At these times chambers were equipped with sensors to measure chamber air temperature, relative humidity, incident PAR, and CO_2 concentration using a LI-COR Model 6200 Portable Photosynthesis System (LI-COR, Inc., Lincoln, NB). After sealing the chambers, several replicate determinations of CO_2 exchange were made within a 10-minute period. Net CO_2 exchange was calculated from changes in CO_2 concentrations within

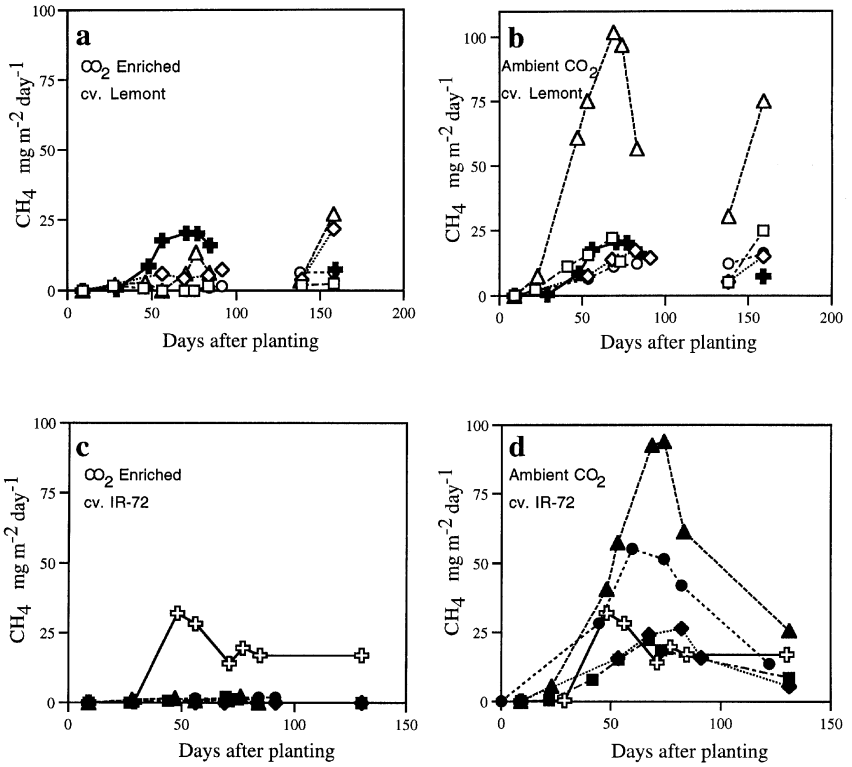

Fig. 4 Seasonal comparison of methane flux values of cv. Lemont rice (a & b) and cv. IR-72 rice (c & d) in each cell along the temperature gradient in the CO_2 enriched tunnel (a & c) and the ambient CO_2 tunnel (b & d), including values from rice grown in the open (outdoor) cell (crosses). Triangles are from Cell 1 (2 °C above ambient), circles from Cell 2, diamonds from Cell 3, and squares from Cell 4 (5 °C above ambient). Error bars are omitted for clarity.

10 ppm of the ambient CO_2 level in a given tunnel. CO_2 levels were maintained within chambers by addition of tank CO_2 between measurements. At a given vat, CO_2 exchange was first measured at ambient sunlight levels. Subsequently, CO_2 exchange was measured at light levels decreasing to total darkness. This was accomplished using 3 levels of screening and a blackout shroud. Chambers were vented with ambient air between each series of measurements at a given light level.

The exchange measured using this system is the net ecosystem exchange of CO_2 (NEE) for the total system which consists of the photosynthesis and respiration of the above-ground biomass, microbial soil respiration and respiration of below-ground plant tissues. NEE is a measure of the productivity of the system, and is equivalent to net primary production minus soil microbial respiration. Rates for both CH_4 and CO_2 were expressed as amount of emission or exchange per ground surface area.

Plant biomass

Both cultivars were sampled for root biomass at 40 and 68 DAP, and cv. IR-72 plants were also sampled at 138 DAP. Pots were removed at these times and above-ground plant material was clipped. Areas outside of the gas exchange collar were sampled. The soil cores were washed to isolate the bulk of the root material, and the water retained. Finer root material floated to the surface and was then removed by hand from the water. All root material from a given pot was combined, dried, and weighed to yield a total root dry weight value for each vat. The above-ground plant material clipped from pots removed at 40 and 68 DAP was separated into stem, leaf, and if present, panicle material (grain-bearing stems), then dried and weighed separately. These values were summed for each vat to give a dry weight value for total above-ground biomass. A partial measurement of above-ground biomass was also taken at a third point in the season (99 DAP for cv. Lemont, 130–131 DAP for cv. IR-72).At these times all panicles found in three rows within the collars were clipped, dried, and weighed to provide a final comparison of above-ground biomass.

Results

Methane flux from all 16 vats in each tunnel and those in the open cell were sampled on a regular basis for an entire growing season. Plants of the Lemont cultivar were also sampled twice following ratooning. Most treatments exhibited a measurable methane flux from the first sampling, which ranged from 21 to 53 DAP. Methane emission showed a single maximum between 60 and 80 DAP (Fig. 4). These generalizations

Table 1 Results of 3-factor ANOVA on methane data treating each week of sampling as an independent measure. All values through week 18 indicate a significant effect of CO_2 treatment to greater than 99% confidence limits with methane fluxes greater from the ambient CO_2 treatment. The CO_2 treatment did not result in a significant effect for the first sampling of cv. Lemont plants after ratooning (19 weeks after planting). There was a significant CO_2 treatment effect (> 95% confidence limit) during the second sampling after ratooning at 22 weeks

Weeks after planting	Significance of CO_2 treatment
4	0.001
7	0.009
8	0.000
10	0.000
11	0.000
12	0.000
14	0.000
18	*0.009
19	†0.231
22	†0.018

Only cv. IR-72 plants were sampled at this time.
† Only ratooned cv. Lemont plants were sampled at this time.

exclude four vats (52–3, 53–3, 53–4, and 54–4) in the CO_2 enriched tunnel with exceptionally low or unmeasurable emission rates throughout the span of this study.

Results from this study show methane emissions from both cultivars to be overwhelmingly lower for plants grown under conditions of CO_2 enrichment compared to plants grown under ambient levels of CO_2 in both the ambient tunnel and the Open Cell (Fig. 4, Table 1). During the regular growing season CH_4 emissions from cells in the CO_2 enriched tunnel were from about 4–45 times lower than corresponding cells in the ambient CO_2 tunnel when compared using the seasonal values. This difference was found to be significant to the 99% confidence levels for each regular season sampling date using a 3-factor ANOVA of independent measures. After ratooning of cv. Lemont plants, methane emission differences between tunnels were not as definitive (Fig. 4). At the first sampling after ratooning, the difference between CO_2 treatments was not significant. By the second sampling of the ratooned crop, average emissions from Cell 3 in the CO_2 enriched tunnel were higher than for the ambient CO_2 tunnel. However, analysis by 3-factor ANOVA showed the overall difference between tunnels to be significant to the 95% confidence levels. Emissions in the ambient tunnel generally were higher than those in the CO_2 enriched tunnel, but the difference between CO_2 treatments had decreased as compared to the regular season (Fig. 4, Table 1).

For both cultivars of rice, methane emissions increased with the increase in temperature from the Open Cell (ambient temperature) to the first cell of the ambient CO_2 tunnel (ambient temperature + 2 °C) (Figs 4 and 5). Within both tunnels and for both cultivars methane emissions decreased with increasing temperature (from 2° to 5 °C above ambient). This was seen most clearly with cv. IR-72 plants (Fig. 5d) and to a lesser extent with cv. Lemont plants grown in the ambient CO_2 tunnel (Fig. 5b). Regression of seasonal emissions plotted against cell number for cv. IR-72 plants grown in the ambient CO_2 tunnel indicated a linear relationship to 95% confidence levels (Fig. 5d).

The CO_2 exchange results revealed a trend opposite that of methane (Table 2). Overall the NEE values for plants grown in the CO_2 enriched tunnel were higher than those for corresponding plants grown in the ambient CO_2 tunnel. This difference was found to be significant to the 95% confidence levels using a 3-way ANOVA of the entire data set. There were no identifiable temperature trends for CO_2 exchange.

Root biomass was higher in the CO_2 enriched tunnel than in the ambient CO_2 tunnel (Table 3). Using a repeated measures 3-factor ANOVA on the data from the first two harvests, the effect of CO_2 enrichment on root biomass was significant to 95% confidence levels. The third root harvest was excluded from this analysis as it appeared that the pots had constrained root growth. Temperature did not have a significant effect on root biomass.

Comparable trends were observed for above-ground biomass results, with each of the three harvests analysed individually using a 3-way ANOVA. On a dry weight basis, above-ground biomass was highest for cv. IR-72 plants at all three harvests (Table 4). For the second and third harvests the effect of increased CO_2 was significant to 95% confidence levels with plants in the CO_2 enriched tunnel having the highest levels of above-ground biomass.

Discussion

This study tested the hypothesis that the combined effect of increased temperature and carbon dioxide concentration would generate a double positive feedback on methane emissions from *Oryza sativa* and that corresponding increases would be observed in NEE and plant biomass. The driving mechanism proposed was that, as a result of these temperature and CO_2 increases, plant productivity and methanogenic activity would be stimulated leading to higher methane emissions. The findings of these experiments did not support this hypothesis in terms of methane emissions. Methane emissions decreased under doubled CO_2. Biomass and NEE para-

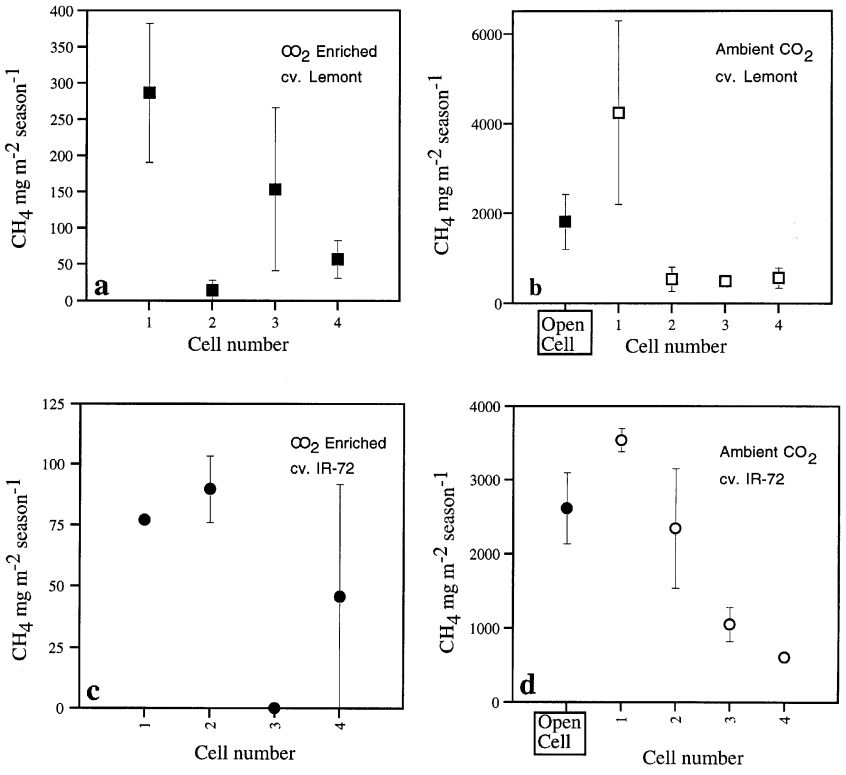

Fig. 5 Average seasonal values (total emissions for the first 89 days after planting) for cv. Lemont plants (a & b) and cv. IR-72 plants (c & d) in the CO_2 enriched tunnel and the ambient CO_2 tunnel. Values are plotted against cell number (i.e. increasing temperature). Cell 1 was 2 °C above ambient and Cell 4 was 5 °C above ambient. The closed symbols in graphs b & d indicate the seasonal value (total emissions for the first 89 days after planting) for the vats from the open cell (ambient temperature) with corresponding cultivar. See Fig. 2 for vat code.

meters, however, did increase in response to increased levels of CO_2. As a function of temperature, CH_4 emissions increased in both cultivars over the 2° transition from the open (ambient) cell to cell 1 within the ambient CO_2 tunnel (Fig. 5). However, with further temperature increase, down both tunnels, methane emissions fell.

The results presented here unequivocally support the conclusion that, during this study, methane emissions from *Oryza sativa* (cvs. Lemont and IR-72) plants grown under conditions of elevated CO_2 were dramatically reduced relative to plants grown in comparable conditions under ambient levels of CO_2. At no time during the regular growing season did emissions from any plot grown in the CO_2 enriched tunnel even approach that from any corresponding plot in the ambient CO_2 tunnel (Fig. 4). These results were replicated in a second year of sampling within this system the following summer when a new rice crop was grown (L.H. Allen, unpubl. data).

One explanation for the observations made during this study is an increased delivery of oxygen to the rhizosphere of plants exposed to the higher CO_2 concentration. This could influence methane emissions in two ways. First, increased O_2 levels in the rhizosphere could support a greater degree of rhizospheric methane oxidation (Bosse & Frenzel 1998; Gilbert *et al.* 1998) effectively diminishing the amount of methane available for transport to the atmosphere. Rhizospheric methane oxidation rates were determined (Schrope 1995) using a methyl fluoride technique (Epp & Chanton 1993), but the results indicated that in the absence of oxidation, methane emissions from the CO_2 enriched tunnel would still not approach those of the ambient CO_2 tunnel. The methyl fluoride technique is not without problems however, so these results may not be definitive (Janssen & Frenzel 1997; Lombardi *et al.* 1997; Van der Nat & Middleburg 1998). We cannot rule out higher methane oxidation in the CO_2 enhanced systems.

A second, and more plausible explanation, is that increased delivery of oxygen to the rhizosphere reduced methane production. The establishment of anaerobic conditions in the rhizosphere could have been suppressed in the CO_2 enriched tunnel if oxygen delivery was higher through the early stages after flooding, and remained high. The Arredondo fine sand used in this experiment is typically 92.4% sand, and only 4.5% and 3.1% silt and clay, respectively (Thomas *et al.* 1985). This high percentage of sand would have allowed for higher rates of O_2 diffusion in the soil. While typical in Florida, this level of sand would normally not be found in other rice agricultural soils. Additionally, the low level of organic matter in the soil (0.5–0.7%, see above) created a small oxygen demand, increasing the O_2 level in the rhizosphere.

CO_2 enriched tunnel		Ambient CO_2 tunnel	
Vat #	NEE ± s.d. mmol CO_2 $m^{-2}d^{-1}$	Vat #	NEE ± s.d. mmol CO_2 $m^{-2}d^{-1}$
Round 1 (22–29 DAP)			
51-1 & 2	32.9 ± 1.1	61-1 & 2	12.8 ± 3.0
51-3 & 4	24.5 ± 5.2	61-3 & 4	13.8 ± 3.3
54-1 & 2	22.2 ± 9.9	64-1 & 2	14.1 ± 2.8
54-3 & 4	21.7 ± 1.6	64-3 & 4	13.8 ± 0.8
Round 2 (41-50 DAP)			
51-1 & 2	18.3 ± 2.6	61-1 & 2	14.7 ± 1.3
51-3 & 4	25.6 ± 6.6	61-3 & 4	20.6 ± 10.7
54-1 & 2	23.1 ± 7.8	64-1 & 2	29.1 ± 1.1
54-3 & 4	40.4 ± 0.7	64-3 & 4	22.9 ± 10.0
Round 3 (73-77 DAP)			
51-1 & 2	8.5 ± 1.4	61-1 & 2	21.1 ± 2.6
51-3 & 4	67.7 ± 1.8	61-3 & 4	9.9 ± 4.5
54-1 & 2	69.1 ± 0.3	64-1 & 2	10.1 ± 1.6
54-3 & 4	69.1 ± 0.3	64-3 & 4	4.7 ± 6.0
Round 4 (91-99 DAP)			
51-1 & 2	11.4 ± 2.0	61-1 & 2	13.4 ± 3.2
51-3 & 4	11.8 ± 0.4	61-3 & 4	5.4 ± 1.0
54-1 & 2	68.6 ± 4.7	64-1 & 2	2.9 ± 3.0
54-3 & 4	16.7 ± 11.2	64-3 & 4	3.9 ± 0.3

Significance of tunnel treatment=0.033

Table 2 Average net ecosystem exchange (NEE) in mmol CO_2 $m^{-2}d^{-1}$ for vats of like cultivar and treatment with standard deviations. See Fig. 2 for vat code. The significance of tunnel treatment (CO_2 enriched vs. ambient CO_2) shown is the result of a 3-way ANOVA of the entire CO_2 exchange data set and indicates a higher NEE in the CO_2 enriched tunnel

Greater O_2 delivery would affect the development of the methanogenic community which requires strict anaerobic conditions (Zinder 1993). Many methanogens can survive brief periods of oxygen exposure lasting for more than 24 h (Kiener & Leisinger 1983), but the changes in oxygen delivery in the present study would have spanned the majority of the growing season. We suggest that the increased O_2 delivery shifted the rhizospheric community to enhance the growth of facultative anaerobes and aerobic microbes, attenuating methane production.

The O_2 delivery to the rhizosphere in the CO_2 enriched tunnel may have been greater relative to the ambient CO_2 tunnel due to the higher root biomass (Table 2). This difference would increase the surface area exposed to soil for flux of oxygen out of the root air spaces. However, to explain the dramatic differences observed in methane emissions, it is likely that increased root biomass would have to be coupled with a particularly well-developed aerenchyma system (as determined by root porosity), because it is this factor that ensures efficient exchange of oxygen with the soil (Kludze *et al.* 1993).

In general changes below ground were the most pronounced with root dry weight increases up to 83% in the CO_2 enriched tunnel relative to the ambient CO_2 tunnel (Table 2). Above-ground differences were also significant, though the differences between tunnels were not as great (up to 35% higher in the CO_2 enriched tunnel) as below-ground biomass differences (Table 3). Similar observations have been made during other studies (Rogers *et al.* 1994; Imai *et al.* 1985; Oechel & Strain 1985).

Roots of flooded rice plants are known to have a high degree of root porosity (Das & Jat 1977; Justin & Armstrong 1987). Das & Jat (1977) found a significant correlation between increasing root porosity and increasing root dry weight for rice plants. The data from this study indicates that root dry weight was in fact higher in the CO_2 enriched tunnel than in the ambient CO_2 tunnel (Table 2). Harvest results suggest that differences persisted throughout the normal growing season, so any differences established in the CO_2 enriched tunnel microbial community due to differences in root biomass or root porosity should have persisted.

The temperatures established in this study were elevated relative to ambient values. This may be of particular importance, because Varade *et al.* (1971) found that for rice, root porosity increases significantly with increasing temperature. No research has been conducted to determine if increased levels of CO_2 stimulate greater root porosity. If such changes in root porosity did result from increased temperature and CO_2 level, the volume of oxygen in the root system would have increased, which would in turn have further increased the quantity of oxygen which could be transported to the rhizosphere.

Table 3 Root Dry Weights for both tunnels and the Open Cell. Numbers above each dry weight column indicate the days after planting (DAP) that the pots were removed and analysed. Only IR-72 plants were harvested three times. See Fig. 2 for vat code. The significance of tunnel treatment (CO_2 enriched vs. ambient CO_2) shown is the result of a 3-way ANOVA treating the first two root harvests as repeated measures and indicates that overall root dry weight was higher in the CO_2 enriched tunnel

Vat number	Root Dry Weight (g)			Vat number	Root Dry Weight (g)		
	40	68	138		40	68	138
CO_2 enriched tunnel				*Ambient CO_2 tunnel*			
51–1	1.38	2.91		61–1	1.17	2.98	
51–2	1.30	5.12		61–2	1.74	2.20	
51–3	2.07	2.03	2.64	61–3	1.28	2.22	3.40
51–4	1.21	8.25	3.23	61–4	1.55	2.79	0.87
52–1	1.64	2.15		62–1	1.28	2.16	
52–2	0.73	4.94		62–2	0.68	3.96	
52–3	1.59	4.30	2.56	62–3	2.55	4.76	5.24
52–4	2.01	7.81	4.00	62–4	1.74	5.71	1.20
53–1	2.76	8.52		63–1	3.35	0.73	
53–2	1.93	4.63		63–2	0.71	1.61	
53–3	3.24	4.61	4.96	63–3	1.46	2.32	9.68
53–4	1.92	4.25	1.52	63–4	0.70	1.00	4.37
54–1	1.64	4.18		64–1	2.15	5.33	
54–2	1.83	4.73		64–2	3.07	1.30	
54–3	3.26	5.91	9.09	64–3	2.89	7.19	3.59
54–4	6.45	6.01	7.36	64–4	1.11	5.20	2.08
Means for cv. Lemont -Tunnel 5				Means for cv. Lemont -Tunnel 6			
*at 40:	1.65 ± 0.58			*at 40:	1.77 ± 1.02		
*at 68:	4.65 ± 1.88			*at 68:	2.53 ± 1.51		
Means for cv. IR-72 -Tunnel 5				Means for cv. IR-72 -Tunnel 6			
*at 40:	2.72 ± 1.67			*at 40:	1.66 ± 0.73		
*at 68:	5.40 ± 2.04			*at 68:	3.90 ± 2.12		
*at 138:	4.42 ± 2.60			*at 138:	3.80 ± 2.82		
Open Cell							
Open 1	1.31	2.63					
Open 2	1.53	6.45					
Open 3	1.20	5.79	5.50				
Open 4	0.95	2.88	1.31				
Means for cv. Lemont -Open Cell							
*at 40:	1.42 ± 0.16						
*at 68:	4.54 ± 2.70						
Means for cv. IR-72 -Open Cell							
*at 40:	1.08 ± 0.18						
*at 68:	4.34 ± 2.06						
*at 130:	3.41 ± 2.96						

*Significance of tunnel treatment = 0.005

We hypothesize that an effect of CO_2 enrichment was enhanced oxygen delivery to the flooded soil and rhizosphere. Indirect evidence for this hypothesis was observed in data collected on the ratooned crop. By cutting the above-ground biomass the process of ratooning may have reduced the supply of O_2 to the rhizosphere by reducing photosynthesis. Anaerobic conditions may have resulted as conditions remained waterlogged, though not completely flooded, during the two week period after ratooning when vats were partially drained to prevent damage to the new crop. This soil state might have allowed the growth of a previously suppressed methanogenic community. It is clear that the difference in methane emissions between tunnels was dramatically reduced after ratooning. Seasonal values from cv. Lemont plants in the CO_2 enriched tunnel were from 10 to 37 times lower than those in the ambient CO_2 tunnel during the regular season. By the second sampling of the ratooned crop (159 DAP; 52 days after clipping) methane emissions from cells 1, 2, and 4 in the CO_2 enriched tunnel were 2.8, 2.5, and 10 times lower (respectively) than corresponding cells in the ambient CO_2 tunnel. Cell 3 emissions for the CO_2 enriched tunnel were slightly higher than in the

Vat Number	Above-ground biomass Dry weight (g) 40–41	68–69	99–130	Vat Number	Above-ground biomass Dry weight (g) 40–41	68–69	99–130
CO_2 enriched tunnel				*Ambient CO_2 tunnel*			
51-1	8.8	14.4	848.9	61-1	7.0	24.1	843.6
51-2	8.5	20.8	790.2	61-2	7.3	15.3	822.7
51-3	9.4	14.6	1111.8	61-3	9.8	21.2	702.5
51-4	15.0	28.3	1203.3	61-4	10.6	22.	775.6
52-1	7.2	12.6	727.7	62-1	8.2	12.87	845.6
52-2	6.6	27.2	840.7	62-2	7.3	27.1	742.3
52-3	9.2	23.7	1028.9	62-3	12.0	29.2	1113.7
52-4	9.4	32.0	1049.0	62-4	13.8	32.0	1063.9
53-1	14.0	34.8	897.3	63-1	9.9	7.7	725.3
53-2	8.8	20.6	721.9	63-2	5.9	12.6	588.0
53-3	13.1	28.0	1104.6	63-3	10.8	17.9	1071.2
53-4	9.3	25.6	821.1	63-4	8.5	13.5	1052.9
54-1	8.8	16.9	774.8	64-1	7.3	16.2	509.1
54-2	8.4	20.7	740.7	64-2	10.9	8.0	548.3
54-3	14.0	30.2	1022.8	64-3	13.2	26.7	908.6
54-4	18.6	26.0	841.8	64-4	9.5	22.0	888.1
Means for cv. Lemont -Tunnel 5				Means for cv. Lemont -Tunnel 6			
*at 40–41:	8.9 ± 2.2			*at 40–41:	8.0 ± 1.6		
*at 68–69:	21.0 ± 7.2			*at 68–69:	15.5 ± 7.0		
*at 99:	792.8 ± 64.0			*at 99:	703.2 ± 136.9		
Means for cv. IR-72 -Tunnel 5				Means for cv. IR-72 -Tunnel 6			
*at 40–41:	12.3 ± 3.5			*at 40–41:	11.1 ± 1.8		
*at 68–69:	26.0 ± 5.3			*at 68–69:	23.1 ± 6.0		
*at 130:	1023.0 ± 131.7			*at 130:	947.1 ± 152.2		
Open Cell							
Open 1	11.7	9.4	678.5				
Open 2	8.3	24.1	620.6				
Open 3	10.3	18.0	781.9				
Open 4	14.2	9.3	859.9				
Means for cv. Lemont -Open Cell							
*at 40–41:	10.0 ± 2.3						
*at 68–69:	16.8 ± 10.4						
*at 99:	649.6 ± 40.9						
Means for cv. IR-72 -Open Cell							
*at 40–41:	12.2 ± 2.7						
*at 68–69:	13.7 ± 6.1						
*at 130:	820.9 ± 55.2						

Table 4 Above-ground biomass on a dry weight basis for both tunnels and the Open Cell. Numbers above each dry weight column indicate the number of days after planting that biomass was harvested. The third harvesting occurred 99 days after planting for cv. Lemont and 130 days after for cv. IR-72. The first two harvests included analysis of above-ground biomass in removable pots. The third harvest included all panicles in three complete rows of rice, and as such can not be compared with the first two. The significance of tunnel treatment (CO_2 enriched vs. ambient CO_2) shown are the result of a 3-way ANOVA treating each harvest as an independent measure. Only the second and third harvests exhibited a significant tunnel effect with above-ground biomass higher in the CO_2 enriched tunnel

Significance of tunnel treatment: *at 40–41 = 0.211; *at 68–69 = 0.049; *at 130 = 0.054

ambient CO_2 tunnel. This dramatically reduced difference between methane emissions from the two tunnels following ratooning provides indirect evidence that oxygen delivery was a factor which suppressed methane production in the CO_2 enriched tunnel.

In order to assess the validity of the increased oxygen delivery hypothesis proposed, further research should be conducted. Using an experimental design similar to this study, redox potential and root porosity should be examined. Redox potentials (expressed in terms of Eh) offer a quantitative measurement of the capacity of the system to donate electrons, which indicates the degree of anaerobiosis of a soil (Farooqui & deMooy 1983). Such measurements would allow a general comparison of oxygen delivery in each tunnel. Root porosity (% air space) values offer another measure of the effect of increased carbon dioxide on potential oxygen delivery. In addition, such measurements would allow the effects of the various characteristics of this study on root porosity to be contrasted with other published results.

Our results provide evidence that the temperature treatment of the first cell in the ambient CO_2 tunnel (ambient +2 °C) was near the optima for the methanogens present. The observed trend in the tunnels was a decrease in methane emissions with increased temperature (Fig. 5). However, when including results from the Open Cell (ambient temperature) with the ambient CO_2 tunnel results (Fig. 5b, d) an increase in methane emissions is observed from ambient temperatures to 2 °C above ambient.

Two studies have reported similar decreases in methane production with increases in temperature above a certain point. Sass *et al.* (1991) examined methane production in soil cores at temperatures from 1 to 65 °C. They found an optimum for production at 37 °C with a decline beyond this point. Similarly, Parashar *et al.* (1993) conducted field experiments with soil temperatures ranging from 26 to 37 °C. They found that methane emissions increased for soil temperatures up to 34.5 °C but decreased sharply above 34.5 °C. The majority of the known methanogens are mesophilic with a temperature optima of about 35 °C (Oremland 1988). Our observations are in accordance with these studies as the lowest temperature treatment in the tunnels (ambient +2 °C) ranged from about 32–43 °C. This appears to have yielded soil temperatures near the temperature optimum for the particular methanogen population found in these soils.

Sass *et al.* (1991) found that the decrease in methane emissions above 37 °C was more rapid than the increase in methane emissions up to 37 °C, but their core results show less effect than was observed for IR-72 vats in the ambient CO_2 tunnel (Fig. 5). This may indicate that more than a simple metabolic stress was taking place in the methanogen population. One possibility would be that stress to plants at such extreme temperatures begins to diminish methane emissions. NEE results did not, however, conclusively indicate such an effect. Another potential explanation is that above a certain temperature increasing root biomass leads to a great enough increase in oxygen delivery such that methane production is attenuated. At the first harvest a significant temperature effect was found with increased temperature leading to increased root biomass (Table 2). Again, further research is required before conclusions can be drawn. Probably a greater temperature gradient needs to be established to include lower temperatures in conjunction with temperatures similar to those used in this study. Baker *et al.* (1994) reported reduced grain yield in rice at elevated temperatures.

It should be noted that the technique employed for sampling of biomass, although necessary to allow normal growth of plants, is in some ways problematic. The relatively small size of pots may hinder the normal growth of roots, particularly in the later stages of growth which could explain why differences between tunnels decreased in IR-72 vats with each successive harvest (Table 3).

Conclusions

The results of this study did not support the our hypothesis that an effect of both increased carbon dioxide and temperature would be an increase in methane emissions. Instead, the opposite was observed. Both increased carbon dioxide (to 700 ppm) and increased temperatures (above 2–5 °C above ambient) were observed to produce decreased methane emissions. However, the change in temperature from the ambient Open Cell to the first cell of the ambient CO_2 tunnel (2 °C above ambient) was observed to produce an increase in methane emissions. Both above- and below-ground, an increased level of carbon dioxide was observed to produce significant increases in biomass. The greatest increases were observed for root biomass. In addition, rates of CO_2 exchange were observed to be higher in response to an increased level of CO_2.

The proposed mechanism for the CO_2 effect on methane emission was that, due to CO_2 enrichment, oxygen delivery to the rhizosphere was increased. This may have occurred by means of an increase in root biomass coupled with a more aerenchymous root tissue relative to roots grown under ambient CO_2 conditions. The inhibitory effect of the higher temperatures was probably a combination of stress to the methanogens as well as to the plants. Unlike the carbon dioxide results, the effect of decreased methane emissions at high temperatures (above 35 °C) has been observed in other studies (Sass *et al.* 1991; Parashar *et al.* 1993).

We hypothesize that the relatively low level of organic matter in the Arredondo fine sand used was a key factor in determining our results. This soil typically contains less than 2% organic matter (Thomas *et al.* 1985). Our own analysis of organic matter levels in the vats are in agreement with this value. By comparison, soil used in the Allen *et al.* (1994) study used lake sediment with 4.3% organic matter and wetland soils typically have high concentrations of organic matter.

The effects of carbon dioxide enrichment observed in this study may not apply to natural wetlands. There the higher level of organic carbon, and long-established anaerobic community should overcome any effect of increased carbon dioxide, as seen in the results of Dacey *et al.* (1994) and Hutchin *et al.* (1995). Likewise, under different conditions, other rice systems may not produce the effects observed in this study (Allen *et al.* 1994). However, the results of this experiment may suggest that

in systems where carbon dioxide enrichment does lead to increased methane emissions, the magnitude of this effect may be dampened by an increase in oxygen delivery to the rhizosphere.

The temperature effects observed in this study are likely to apply to most wetland systems (natural and agricultural) with temperate to subtropical climates. The effects of any increases in methane emissions resulting from increased levels of carbon dioxide may be partially balanced if coupled with temperature increases greater than 2 °C. Our results indicate that temperature changes less than 2 °C have the effect of enhancing methane emissions.

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